[00241] **Example 9**

[00242] **RESTORATIVE EFFECTS OF ERYTHROPOIETIN ON DIMINSHED COGNITIVE FUNCTION ARISING FROM BRAIN INJURY**

[00243] In a study to demonstrate the ability of erythropoietin to restore diminished cognitive function in mice after receiving brain trauma, female Balb/c mice were subject to blunt brain trauma as described in Brines et al. PNAS 2000, 97; 10295-10672 and five days later, daily erythropoietin administration of 5000 U (40 μg)/kg-bw intraperitoneally was begun. Twelve days after injury, animals were tested for cognitive function in the Morris water maze, with four trials per day. While both treated and untreated animals performed poorly in the test (with swim times of about 80 seconds out of a possible 90 seconds), **Figure 18** shows that the erythropoietin-treated animals performed better (in this presentation, a negative value is better). Even if the initiation of erythropoietin treatment is delayed until 30 days after trauma (**Figure 19**), restoration of cognitive function is also seen. Similar results would be expected from treatment with the tissue protective cytokines of the present invention.

[00244] **Example 10**

[00245] **KAINATE MODEL**

[00246] In the kainate neurotoxicity model, asialoerythropoietin was administered according to the protocol of Brines et al. Proc. Nat. Acad. Sci. U.S.A. 2000, 97; 10295-10672 at a dose of 5000U (40 μg)/kg-bw given intraperitoneally 24 hours before the administration of 25 mg/kg kainate is shown to be as effective as erythropoietin, as shown by time to death (**Figure 20**). Similar results are obtainable from treatment with the tissue protective cytokines of the present

invention.

[00247] **Example 11**

[00248] **SPINAL CORD INJURY MODELS**

[00249]

[00250] 1. Rat Spinal Cord Compression Testing Erythropoietin and Tissue Protective Cytokines

[00251] Wistar rats ~~(female) weighing~~ 180–300g were used in this study. The animals were fasted for 12 h before surgery, and were humanely restrained and anesthesized with an intraperitoneal injection of thiopental sodium (40 mg/kg-bw). After infiltration of the skin (bupivacaine 0.25%), a complete single level (T-3) laminectomy was performed through a 2 cm incision with the aid of a dissecting microscope. Traumatic spinal cord injury was induced by the extradural application of a temporary aneurysm clip exerting a 0.6 newton (65 grams) closing force on the spinal cord for 1 minute. After removal of the clip, the skin incision was closed and the animals allowed to recover fully from anethesia and returned to their cages. The rats were monitored continuously with bladder palpation at least twice daily until spontaneous voiding resumed.

[00252] 40 animals were randomly divided into five groups. Animals in the control group (I) (n= 8) received normal saline (via intravenous injection) immediately after the incision is closed. Group (II; n= 8) received rhEPO @ 16 micrograms/kg-bw iv; group (III) received an asialo tissue protective cytokine of the present invention (asialoerythropoietin) @ 16 micrograms/kg-bw iv, group (IV) received an asialo tissue protective cytokine @ 30 micrograms/kg-bw iv, and group (V) received an asialo tissue protective cytokine of the present invention (asialoerythropoietin) @ 30 micrograms/kg-bw; all as a single bolus intravenous injection immediately after removal of the aneurysm clip.

Figure 18

Morris water maze; female Balb/c mice n= 16 . Blunt brain trauma with EPO rx beginning on day 5 after injury. First water maze test began 1 week after EPO dosing began (12 days after injury). Both groups of animals did poorly with swim times ~80 out of 90 seconds possible. Negative values indicates that EPO is better. Means of 4 trials per day.

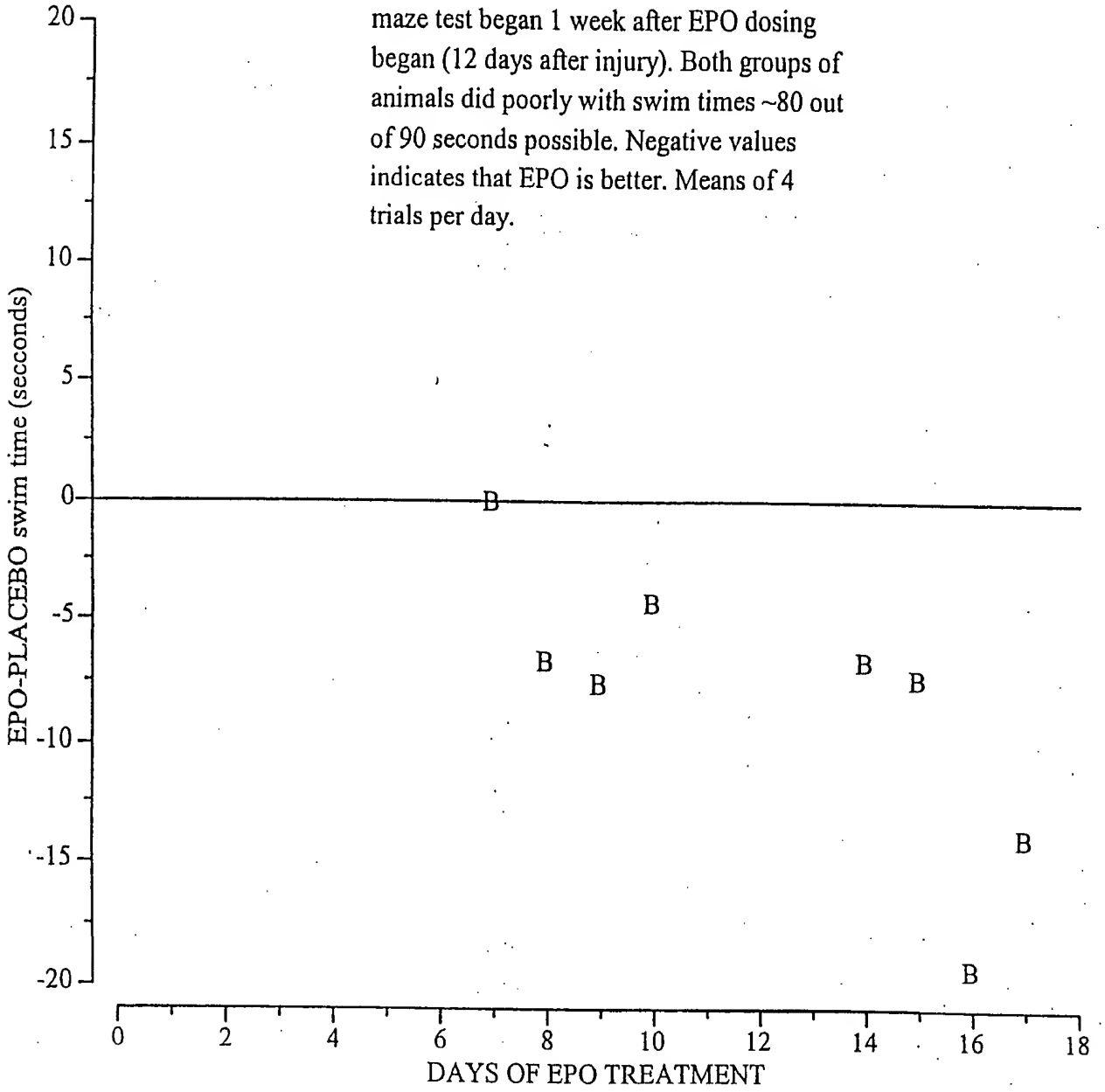


Figure 19

Difference in swimming time to platform in Morris water maze.
Bab/c mice; brain trauma 1 month previously, treated with 5000U/kg EPO daily except weekends
beginning one month after injury
(n= 7 each group)
means of 4 trials each day

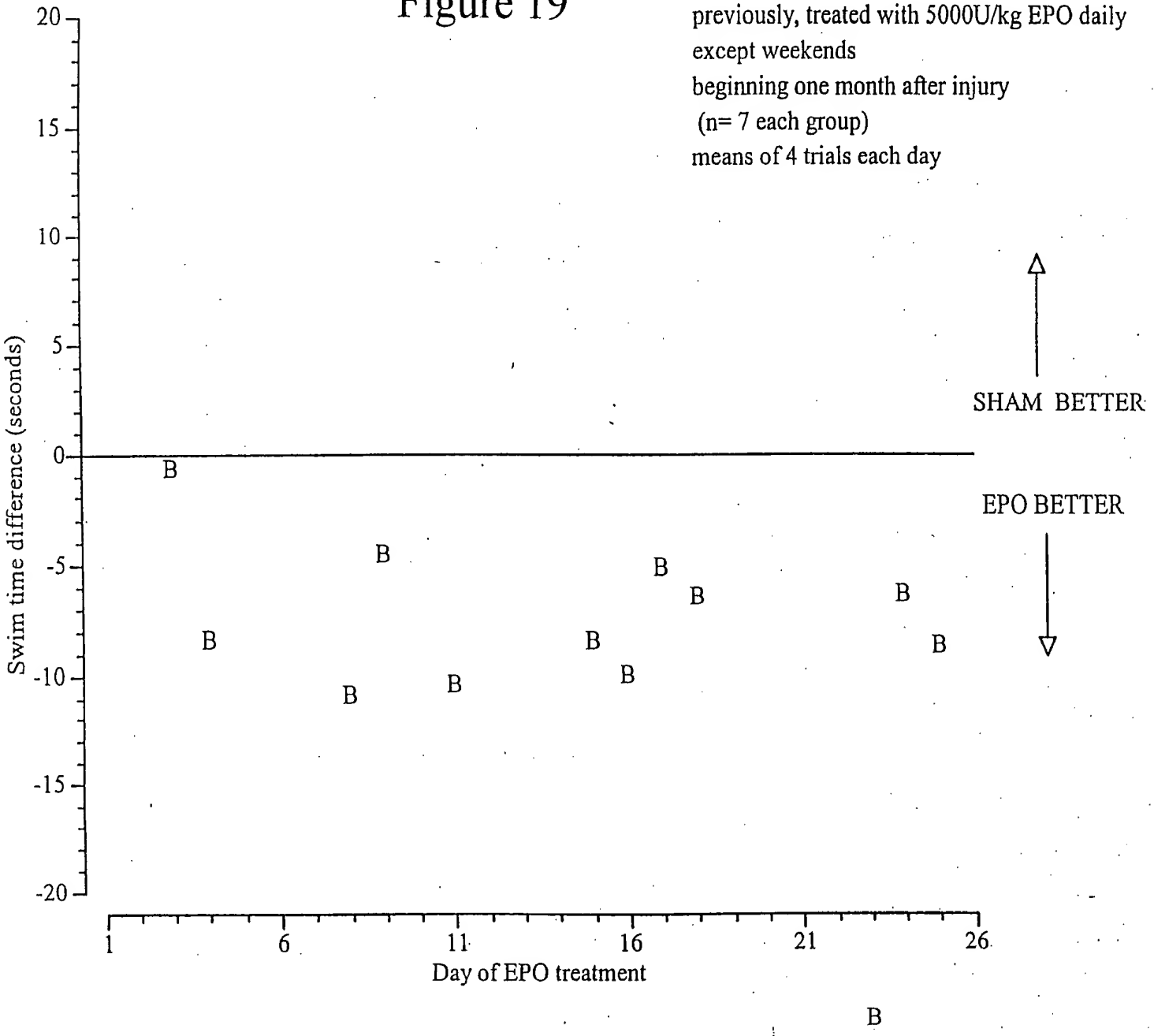
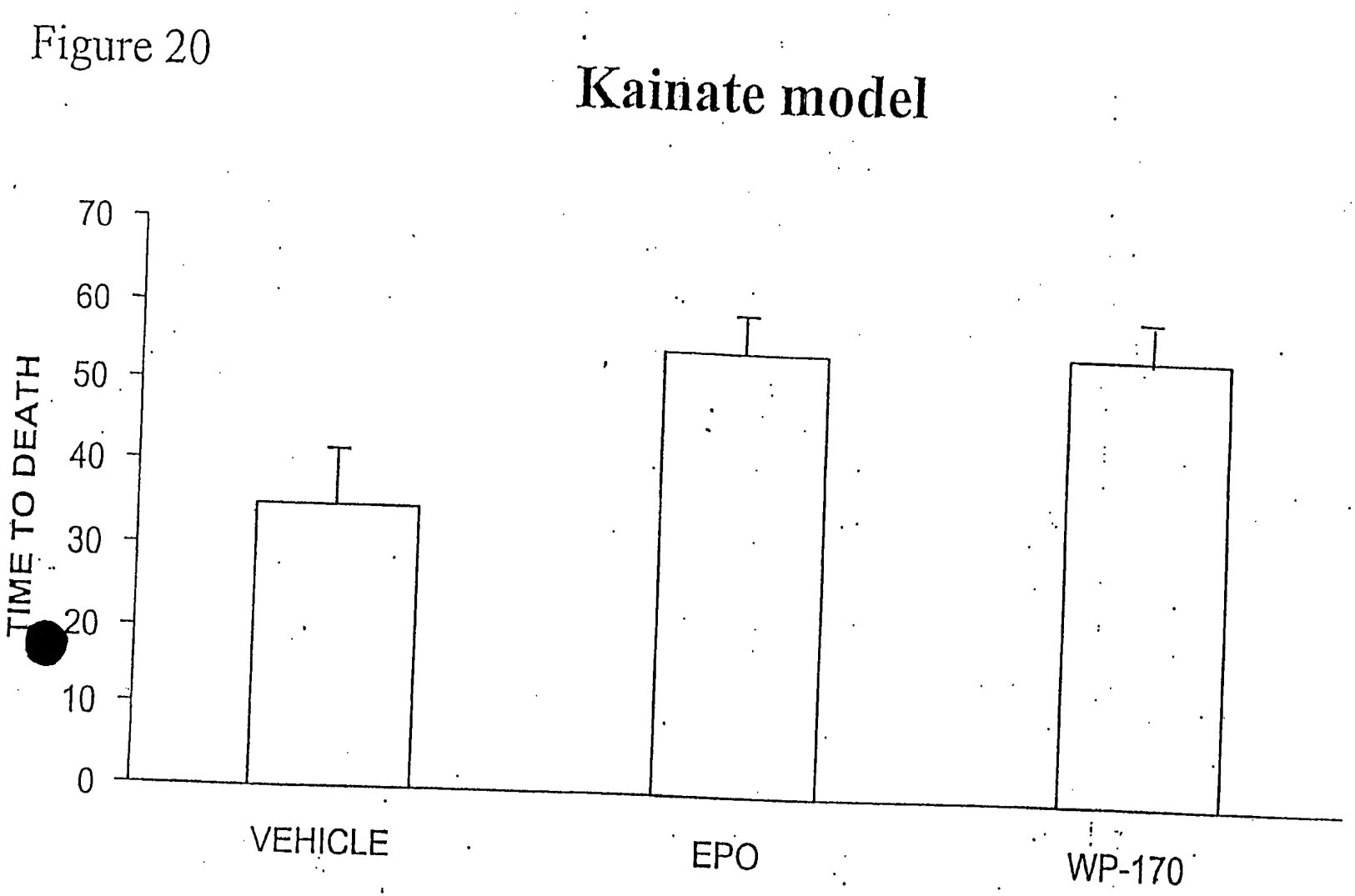


Figure 20


Kainate model
TIME TO DEATH
70
60
50
40
30
20
10
0
VEHICLE
EPO
WP-170